

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
29 December 2004 (29.12.2004)

PCT

(10) International Publication Number
WO 2004/112703 A2

(51) International Patent Classification⁷:

A61K

(21) International Application Number:

PCT/US2004/018903

(22) International Filing Date: 15 June 2004 (15.06.2004)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/479,772

19 June 2003 (19.06.2003) US

(71) Applicant (for all designated States except US): CENTOCOR, INC. [US/US]; 200 Great Valley Parkway, Malvern, PA 19355 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): CUNNINGHAM, Mark, R. [GB/US]; 716 Garden Drive, Kennett Square, PA 19348 (US). HEAVNER, George, A. [US/US]; 6 Oak Glen Drive, Malvern, PA 19355 (US). LUO, Jinquan [CN/US]; 46 Knickerbocker Lane, Malvern, PA 19355 (US). SONG, Xiao-yu, R. [US/US]; 1004 Wiggins Way, West Chester, PA 19380 (US).

(74) Agents: JOHNSON, Philip, S. et al.; One Johnson & Johnson Plaza, New Brunswick, NJ 08933 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: INTERLEUKIN-21 ANALOGS

Native QDRHMIRMRQ LIDIVDQLKN YVNDLVPEFL PAPEDVETNC EWSAFSCFOK AQQLKSANTGN
 #1 N-----E-----V-----Q-----I-----T-----Q-----
 #2 N-----V-----Q-----I-----T-----Q-----
 #3 N-----E-----V-----Q-----I-----T-----Q-----
 #4 N-----E-----V-----Q-----I-----T-----Q-----
 #10A N-----E-----V-----E-----I-----T-----R-----

Native NERIINVSIK KLKRKPPSTN AGRROKHRLT CPSCDSYEKK PPKEFLERFK SJLQKMIHQH LSSRTHGSED S
 #1 -----Q-----T-----N-----T-----Y-----Y-----V-----E-----T-----
 #2 -----Q-----T-----N-----T-----Y-----Y-----V-----E-----T-----
 #3 -----Q-----T-----N-----T-----Y-----V-----E-----T-----
 #4 -----Q-----T-----N-----T-----Y-----V-----E-----T-----
 #10A -----Q-----T-----N-----T-----Y-----V-----E-----

WO 2004/112703 A2

(57) Abstract: Isolated polynucleotides encoding mature interleukin-21, interleukin-21 analogs, polypeptides obtainable from the polynucleotides and uses are disclosed.

5

INTERLEUKIN-21 ANALOGS**Field of the Invention**

The present invention relates to mature interleukin-21, interleukin-21 analogs and the polynucleotides encoding them.

10

Background of the Invention

Cytokines, the family of bioactive proteins and polypeptides synthesized by white blood cells and virtually all other nucleated cells, are secreted in response to microbes and other antigens, as well as environmental stimuli. They mediate diverse biological processes that are required for the maintenance of homeostasis and host defense. These processes include immune responses, inflammation, cell growth, tissue repair, fibrosis and angiogenesis. Cytokines play critical roles in host defense against pathogens and provide links between innate and adaptive immunity. They also regulate the magnitude and the nature of immune responses by influencing the growth and differentiation of immune cells.

The cytokine interleukin 21 (IL-21) has been identified by Parrish-Novak *et al.* in *Nature* 408, 57-63 (2000). Expression of human IL21 (huIL-21) in normal tissues was not detectable by Northern analysis. However, upon quantitative RT-PCR analysis, an increase in IL-21 mRNA level was detected in phorbol-12-myristate-13-acetate/ionomycin-activated or anti-CD3-treated human peripheral T cells suggesting that IL-21 may be used by T cells to stimulate effector cell function (Parrish-Novak *et al.*, *supra*). In contrast, no IL-21 expression was detected on CD19⁺ B cells and CD14⁺ monocytes (Parrish-Novak *et al.*, *supra*).

The sequence of hIL-21 cDNA contains an open reading frame that encodes a polypeptide precursor of 162 amino acids. The signal peptidase cleavage rules predict a cleavage site after Gly31. The mature polypeptide is a soluble monomeric non-glycosylated protein with a predicted relative molecular mass of 15 kDa and consists of a 131-residue four-helix-bundle cytokine domain with significant homology to IL-2, IL-4, and IL-15, which also share a common C subunit receptor (Asao *et al.*, *J. Immunol.* 167, 1-5 (2001); Sugamura and Asao, *Adv. Immunol.* 59, 225 (1995); and Grabstein *et al.*, *Science* 264, 965-968 (1994)). huIL-21 and huIL-15 share two pairs of

5 cysteine residues in identical positions, one pair that is conserved in IL-2, IL-4, and granulocyte-macrophage colony-stimulating factor (GM-CSF) and one that is unique to IL-21 and IL-15. This structure consistency, together with the relatively high degree of amino-acid homology, indicates that IL-15 is the closest structural relative of IL-21. In general, cytokines are predicted to have a four-alpha helix structure, with helices A, C
10 and D being most important in ligand-receptor interactions and more highly conserved among members of this family.

In vitro studies showed that IL-21 induces CD34⁺ bone marrow progenitor cell proliferation in combination with IL-15 (Parrish-Novak *et al.*, *supra*). In addition, IL-21 also enhances effector function of the CD56⁺CD16^{bright} cells in the presence of Flt3L
15 and IL-15. Moreover, NK cells cultured with IL-21 exhibit enhanced lytic activity on K562 target cells, although the effect of IL-21 is not quite as pronounced as that of IL-2 or IL-15. IL-21 also stimulates the proliferation of mature B cells when co-stimulated with anti-CD40 and T cells when co-stimulated with anti-CD3. In addition, IL-21 acts in concert with IL-2, IL-15, and IL-7 to enhance T cell proliferation, either with or
20 without anti-CD3 stimulation (Parrish-Novak *et al.*, *supra*). Recent studies showed that IL-21 enhances cytotoxic activity and IFN γ production by activated murine NK cells but does not sustain their viability. Moreover, IL-21 blocks IL-15-induced expansion of resting NK cells. In contrast, IL-21 enhances the proliferation, IFN γ production and cytotoxic function of antigen specific CD8⁺ effector T cells suggesting that IL-21
25 promotes the transition between innate and adaptive immunity (Kasaian *et al.*, *Immunity* 16, 559-569 (2002)). In addition, IL-21 can inhibit antigen-specific and IL-4 induced IgE production and IL-21R deficient mice exhibited higher levels of IgE after immunization as compared with wild type counterparts (Suto *et al.*, *Blood* 100, 4565-4573 (2002); Ozaki *et al.*, *Science* 298, 1630-1634 (2002)). Finally, IL-21 is also a
30 growth and survival factor for human myeloma cells (Brenne *et al.*, *Blood* 99, 3756-3762 (2002)). Therefore, IL-21 seems to play an important role in regulating the immune system and may become a therapeutic target for various immune-mediated inflammatory disorders, allergic disorders, as well as cancers and infectious diseases.
Thus, there is a need to generate a panel of monoclonal antibodies that will recognize
35 different epitopes on IL-21 and could subsequently become potential therapeutics or diagnostics for a variety of diseases or research reagents for discovering new therapies.

5 Novel analogs of huIL-21 can be used for the generation of antibodies that recognize
IL-21. The use of these analogs to generate antibodies by immunization, phage
panning or other techniques will result in antibodies that recognize specific regions of
huIL-21. Analog IL-21 proteins can potentially provide enhanced properties, such as
increased or modified biological half lives, modified biological activities, enhanced
10 immunogenicity for generating antibodies, increased stability or expression, and the
like.

Brief Description of the Drawings

Fig. 1 shows stimulation of human NK-92 cells by a huIL-21 analog and wild type
15 huIL-21.

Fig. 2 shows an amino acid sequence alignment of huIL-21 analogs to the predicted
mature form native sequence of huIL-21.

Summary of the Invention

20 One aspect of the invention is an isolated polynucleotide comprising a polynucleotide
having the sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11 or 13 or a complementary
sequence.

Another aspect of the invention is an isolated polynucleotide comprising a
polynucleotide encoding the amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8, 10,
25 12 or 14 or a complementary sequence.

Another aspect of the invention is an isolated polypeptide comprising a polypeptide
having the sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14.

Another aspect of the invention is an isolated mature huIL-21 having the amino acid
sequence shown in SEQ ID NO: 19.

30 Yet another aspect of the invention is an isolated polynucleotide encoding mature huIL-
21 and having a sequence shown in SEQ ID NO: 18.

Detailed Description of the Invention

All publications, including but not limited to patents and patent applications, cited in
35 this specification are herein incorporated by reference as though fully set forth.

5 The present invention provides isolated analogs of IL-21. In particular, the invention
provides huIL-21 analog polypeptides and polynucleotides. The polypeptides of the
invention are related by amino acid sequence homology to the polypeptides having the
sequences set forth in SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14. The invention especially
provides huIL-21 analogs having the amino acid sequences set forth in SEQ ID NOs: 2,
10 4, 6, 8, 10, 12 or 14 and polynucleotides encoding them including, but not limited to,
polynucleotides having the sequences set forth in SEQ ID NOs: 1, 3, 5, 7, 9, 11 or 13 or
their complementary sequences. The invention further provides for equivalent
fragments and variants of huIL-21 analogs, as well as encoding or complementary
nucleic acids, vectors comprising a huIL-21 analog, host cells containing such vectors
15 and methods of making and methods of use of such analogs, vectors or host cells.

One aspect of the invention provides biologically active variants of huIL-21 useful for
generation and screening of antibodies against IL-21. Anti-IL-21 antibodies are useful
as therapeutic agents, diagnostic agents or research reagents. The present invention
further provides for equivalent isolated polypeptides that: (a) comprise or consist of an
20 amino acid sequence which has at least 95% identity, most preferably 97-99% or exact
identity, to the entire amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12 or
14; (b) is encoded by an isolated polynucleotide comprising or consisting of a
polynucleotide sequence that has at least 95% identity, most preferably 97-99% or
exact identity, to the entire nucleotide sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11
25 or 13; or (c) is encoded by an isolated polynucleotide comprising or consisting of a
polynucleotide sequence encoding a polypeptide which has at least 95% identity, most
preferably 97-99% or exact identity, to the entire amino acid sequence shown in SEQ
ID NO: 2, 4, 6, 8, 10, 12 or 14.

Values for % identity can be obtained from amino acid and nucleotide sequence
30 alignments generated using the default settings for the AlignX component of Vector
NTI Suite 8.0 (Informax, Frederick, MD).

The polypeptides of the invention include a polypeptide having an amino acid sequence
as set forth in SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14 as well as equivalent polypeptides
and fragments that have the biological activity of huIL-21 and have at least 95%
35 identity to a polypeptide having the amino acid sequence shown in SEQ ID NO: 2, 4, 6,
8, 10, 12 or 14 and include portions of the polypeptide generally containing at least 15-

5 50 amino acids. Exemplary embodiments of the invention are the polypeptides having the amino acid sequences set forth in SEQ ID NO: 8, 10, 12 or 14.

In the invention, the polypeptides having the amino acid sequences set forth in SEQ ID NO: 8 or 10 have two additional N-terminal amino acids as set forth in SEQ ID NOs: 12 and 14, respectively. Exemplary nucleotide sequences encoding these polypeptides 10 are set forth in SEQ ID NOs: 11 and 13, respectively. The present inventors have discovered that mature IL-21 does not have a mass corresponding to an N-terminal residue of Gln-32 (as predicted by Novak *et al.* in U.S. Pat. No. 6,307,024) but instead has a mass of 16,815.8 Da corresponding to Gln-30 as the actual N-terminal residue. See Example 2 below. Thus, the actual secretory signal sequence includes amino acid 15 residues 1 (Met) to 29 (Ser) (SEQ ID NO: 17) and the actual mature polypeptide includes amino acid residues 30 (Gln) to 162 (Ser) (residues 1 to 133 in SEQ ID NO: 19). Thus, another aspect of the invention is an isolated mature human interleukin-21 having the sequence shown in SEQ ID NO: 19 and polynucleotides encoding it such as that having the sequence shown in SEQ ID NO: 18.

20 A "fragment" is a variant polypeptide having an amino acid sequence that is entirely the same as part but not all of any amino acid sequence of any polypeptide of the invention. Fragments can include, e.g., truncation polypeptides having a portion of an amino acid sequence as shown in amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8, 10 or 12, or of variants thereof, such as a continuous series of residues that includes a

25 heterologous amino- and/or carboxy-terminal amino acid sequence. Degradation forms of the polypeptides of the invention produced by or in a host cell are also included.

Other exemplary fragments are characterized by structural or functional attributes such as fragments that comprise alpha-helix or alpha-helix forming regions, beta-sheet or beta-sheet forming regions, turn or turn-forming regions, coil or coil-forming regions,

30 hydrophilic regions, hydrophobic regions, alpha-amphipathic regions, beta-amphipathic regions, flexible regions, surface-forming regions, substrate binding regions, extracellular regions and high antigenic index regions.

Further exemplary fragments include an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids from the

35 amino acid sequence set forth in SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14, or an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or

5 100 contiguous amino acids truncated or deleted from the amino acid sequence set forth in amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14.

The present invention further provides for equivalent isolated polynucleotides that comprise or consist of (a) a polynucleotide sequence which has at least 95% identity, most preferably 97-99% or exact identity, to the entire nucleotide sequence shown in

10 SEQ ID NO: 1, 3, 5, 7, 9, 11 or 13; or (b) a polynucleotide encoding a polypeptide sequence that has at least 95% identity, most preferably 97-99% or exact identity, to the entire polypeptide sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14.

The polynucleotides of the invention include a mature polypeptide coding sequence having a nucleotide sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 9, 11 or 13.

15 Exemplary embodiments of the invention are the polynucleotides having the nucleotide sequences set forth in SEQ ID NO: 7, 9, 11 or 13.

The invention also provides a mature polypeptide coding sequence or a fragment thereof in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre- or pro- or prepro-protein sequence. The 20 polynucleotides of the invention may also contain at least one non-coding sequence, such as transcribed but not translated sequences, termination signals, ribosome binding sites, Kozak sequences, mRNA stabilizing sequences, introns and polyadenylation signals. The polynucleotide sequences may also contain additional sequences encoding additional amino acids. These additional polynucleotide sequences may, for example, 25 encode a marker sequence such as a hexa-histidine peptide, as described in Gentz *et al.*, *Proc. Natl. Acad. Sci. (USA)* 86, 821-824 (1989) or the HA peptide tag as described in Wilson *et al.*, *Cell* 37, 767 (1984) which facilitate the purification of fused polypeptides. Polynucleotides of the invention can also include structural gene polynucleotides and associated gene expression control sequences.

30 The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the invention.

35 For recombinant production of the polypeptides of the invention, host cells can be genetically engineered to incorporate expression systems or portions thereof and

5 polynucleotides of the invention. Introduction of a polynucleotide into a host cell can
be effected by methods well known to those skilled in the art from laboratory manuals
such as Davis *et al.*, *Basic Methods in Molecular Biology*, 2nd ed., Appleton & Lange,
Norwalk, CT (1994) and Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*,
3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (2001). These
10 methods include calcium phosphate transfection, DEAE-Dextran mediated transfection,
transvection, microinjection, cationic lipid-mediated transfection, electroporation,
transduction, scrape loading, ballistic introduction and infection.

Representative examples of hosts include Archaea cells; bacterial cells such as
streptococci, staphylococci, enterococci, *E. coli*, streptomycetes, cyanobacteria, *B.*
15 *subtilis* and *S. aureus*; fungal cells such as *Kluveromyces*, *Saccharomyces*,
Basidomycete, *Candida albicans* or *Aspergillus*; insect cells such as *Drosophila S2* and
Spodoptera Sf9; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293, CV-1,
Bowes melanoma and myeloma; and plant cells, such as gymnosperm or angiosperm
cells.

20 A great variety of expression systems can be used to produce the polypeptides of the
invention. Such systems include chromosomal-, episomal- and virus-derived vectors
such as vectors derived from bacterial plasmids, bacteriophage, transposons, yeast
episomes, insertion elements, yeast chromosomal elements, baculoviruses, papova
viruses such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies
25 viruses, picornaviruses and retroviruses and vectors derived from combinations thereof,
such as cosmids and phagemids. The expression system constructs may contain control
regions that regulate or cause expression. Generally, any system or vector suitable to
maintain or propagate polynucleotides and/or to express polypeptides in a host may be
used for expression. An appropriate DNA sequence may be inserted into the
30 expression system by any of a variety of techniques well known to those skilled in the
art, such as, e.g., those set forth in Sambrook *et al.*, *supra*.

In eukaryotic expression systems, polypeptides of the invention can be secreted into the
lumen of the endoplasmic reticulum or extracellular environment by inclusion of
appropriate secretion signals such as a signal peptide or leader sequence. These signals
35 may be heterologous or endogenous to huIL-21 such as those listed in SEQ ID NO: 15
(predicted) or SEQ ID NO: 17 (actual).

5 The polypeptides of the present invention may also be produced by chemical synthesis such as solid phase peptide synthesis on an automated peptide synthesizer, using known amino acid sequences or amino acid sequences derived from the DNA sequence of the polynucleotides of the invention. Such techniques are well known to those skilled in the art.

10 Polypeptides of the invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, high-performance liquid chromatography, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxyapatite chromatography and lectin chromatography. Well-known techniques for refolding protein may be employed to regenerate an active conformation when the protein is denatured during isolation and/or purification.

15 The polynucleotides and polypeptides of the invention comprising at least one epitope of IL-21 can be used to produce polyclonal or monoclonal antibodies. These analogs may exhibit increased binding efficiency to IL-21 receptor and/or be more immunogenic than wild type IL-21. Techniques for making murine, chimeric, humanized and fully human monoclonal antibodies using protein or nucleic acid immunization are known to those skilled in the art.

20 The polynucleotides and polypeptides of the invention are also useful for assaying a medium for the presence of a substance that modulates IL-21 protein function by affecting the binding of an IL-21 analog protein to cellular binding partners such as the IL-21 receptor. Examples of modulators include polypeptides or small organic molecules.

25 Mature IL-21 or its analogs can be used to modulate, *i.e.*, increase or decrease, immune cell activity and/or number such as the activity and/or number of T cells (CD4⁺, CD8⁺ and mature T cells), NK cells (resting or mature), B cells, dendritic cells, macrophages, megakaryocytes or a population of immune cells. Moreover, mature IL-21 or its analogs can be used to dampen IgE production in allergic diseases as well as asthma and other parasitic diseases mediated by IgE. Since IL-21 can inhibit the production of IFN γ from developing Th1 cells (Wurster *et al.*, *J. Exp. Med.* 196, 969-977, (2002)), mature IL-21 or its analogs can also be used to treat various kinds of immune-mediated

- 5 inflammatory diseases that are dependent on IFN γ such as multiple sclerosis. In addition, mature IL-21 or its analogs can be used to enhance immune responses to a cancer or infectious disease. Mature IL-21 or its analogs can be used alone or in combination with an antigen as an adjuvant to treat or prevent various cancers such as solid tumors, soft tissue tumors (such as lymphoma or leukemia) and metastatic lesions.
- 10 Further, mature IL-21 or its analogs can be used to treat or prevent infectious disorders including bacterial, viral and parasitic disorders.

The mode of administration for therapeutic use of the polypeptides of the invention may be any suitable route which delivers the agent to the host. The polypeptides and their pharmaceutical compositions of these agents are particularly useful for parenteral administration, *i.e.*, subcutaneously, intramuscularly, intradermally, intravenously or intranasally.

Polypeptides of the invention may be prepared as pharmaceutical compositions containing an effective amount of the binding agent as an active ingredient in a pharmaceutically acceptable carrier. An aqueous suspension or solution containing the binding agent, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the binding agent of the invention or a cocktail thereof dissolved in an pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, *e.g.*, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (*e.g.*, filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the polypeptides of the invention in such pharmaceutical formulation can vary widely, *i.e.*, from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, *e.g.* about 50 ng to about 30 mg or more preferably, about 5 mg to about 25 mg, of a polypeptide of the invention. Similarly, a pharmaceutical composition of the

5 invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 mg to about 30 mg and preferably 5 mg to about 25 mg of a polypeptide of the invention. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, *Remington, the Science and*
10 *Practice of Pharmacy*, 19th ed., Mack Publishing Company, Easton, Pa (1995).

The polypeptide of the invention, when in a pharmaceutical preparation, can be present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by those of skill in the art. A determined dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician during the treatment period.
15

The polypeptides of the invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional protein preparations and art-known lyophilization and reconstitution techniques can be employed.

20 The present invention will now be described with reference to the following specific, non-limiting examples.

Example 1**Human IL-21 Analog Design and Testing**

25 A molecular model of huIL-21 was created with the InsightII software suite (Accelrys, San Diego, CA). Each amino acid in the model was examined visually and potential substitutions considered on the basis of retention of hydrophobic, steric and hydrogen-bonding characteristics of the parent amino acids. A molecular model of each proposed analog was made using the same conditions used to create the parent molecule model.

30 These structures were evaluated and structures with any of the following properties were not considered further:

a structure of significantly higher energy as calculated by InsightII;

significant alterations in the backbone structure;

significant changes in the surface exposed residues;

35 significant changes of the hydrophobicity of the surface; or

alterations in hydrogen-bonding patterns.

5 Various combinations of acceptable amino acid substitutions were then modeled further and evaluated as previously described. Of the combinations considered, eight analogs were generated.

DNA encoding each of the eight analogs was obtained synthetically and cloned into a mammalian cell expression vector containing the CMV promoter and the bovine

10 growth hormone polyA transcriptional control sequences and evaluated for their ability to generate a secreted protein. Polypeptides produced by the expression vector contained an N-terminal secretory signal sequence (SEQ ID NO: X) from wild type IL-21 and a C-terminal hexa-histidine tag. Briefly, HEK 293E cells were transfected with the expression vectors using a cationic lipid reagent to facilitate DNA uptake by the

15 cells. After 24 hours, the cells were placed in a serum-free medium formulation and grown for 4 days. Following this period, the conditioned medium was recovered and subjected to immobilized metal affinity chromatography (IMAC) using TALON™ resin (CLONTECH Laboratories, Inc., Palo Alto, CA) to purify His-tagged proteins. Any proteins bound to the column were eluted using EDTA and subjected to

20 Coomassie-stained SDS PAGE and anti-His Western blot. Analysis of visible bands on the SDS PAGE gels and anti-His Western blots indicated that human IL-21 analog #1, 2, 3, and 4 (SEQ ID NOs: 2, 4, 6 and 8, respectively) were secreted (data not shown).

Biological activity of analog #4 (SEQ ID NOs: 7 and 8) was determined in an NK-92 cell proliferation assay. This analog was expressed at 1L scale and purified using

25 IMAC TALON™. Human NK-92 cells were cultured in 96-well flat bottom tissue culture plates (white plate and clear bottom, VWR, Bridgeport, NJ). 100 µL of cells were plated per well at a density of 1X10⁵ cells per ml in MEM (Invitrogen, Carlsbad, CA) supplemented with 12.5% FBS (JRH Bioscience, Lenexa, KS), 12.5% horse serum, 0.2 mM inositol, 0.02 mM folic acid, 100 µM beta-mercaptoethanol (Sigma, St. Louis, MO), 10 ng/ml of hIL-2 and hIL-15 (PeproTech, Rocky Hill, NJ). The cells were cultured in triplicates, washed twice with medium and assayed for proliferation in the presence of serially diluted purified IL-21, IMAC TALON™ purified IL-21 analog #4 or control protein (murine tissue factor variant) for 72 hrs at 37°C, 5% CO₂. Per kit directions (Packard, Boston, MA), the cells were then lysed and ATP-lite substrate was added. Luminescence was measured by using a Topcount plate reader (Packard).

5 The results shown in Fig. 1 indicated that both huIL-21 variant #4 and wild-type protein promote the proliferation of NK-92 cells in a dose dependent fashion. The EC₅₀ for huIL-21 variant #4 is 1.014 ng/ml and that of wild-type huIL-21 protein ranges between 1 ng/ml and 4.6 ng/ml for different lots. Each data point represents the mean ± SD of triplicates.

10 Analog #4 was used as a template for further rounds of variant construction. The original mutations were grouped into four families depending upon surface and internal positioning. For each family, two sets of mutations were made either to revert amino acids back to wild-type sequence or change amino acids to an alternative sequence. Nine variants were expressed and protein secretion characterized as described above.

15 The results (not shown) indicated that huIL-21 analog 10A was expressed and secreted at slightly reduced levels compared to wild-type huIL-21. The amino acid sequence alignment of huIL-21 analog nos. 1, 2, 3, 4 and 10A (SEQ ID NOs: 2, 4, 6, 8 and 10, respectively) compared to the predicted mature form native sequence of huIL-21 are shown in Fig. 2.

20 **Example 2**

SELDI-TOF Mass Spectrometry of huIL-21

Purified recombinant wild-type human IL-21 protein was analyzed by Surface Enhanced Laser Desorption Ionization (SELDI) Time-of-Flight (TOF) mass spectrometry. Briefly, 3 µL of huIL-21 (\geq 0.1mg/mL) was spotted on to a C18 hydrocarbon derivatized LDI-TOF solid sample support H4 chip (Ciphergen Biosystems, Inc., Fremont, CA) and allowed to dry at room temperature. The sample was then washed on the chip three times with 3 µL Milli-Q H₂O (Millipore Corporation, Billerica, MA) and 1 µL of saturated sinapinic acid (Sigma-Aldrich Co., St. Louis, MO) in 50% acetonitrile with 1% trifluoroacetic acid was applied to the IL-21 spot and allowed to dry at room temperature. The mass spectrum was acquired on a SELDI spectrometer (Ciphergen Biosystems, Inc.) to obtain the m/z values for the protein sample. The mass measured for recombinant IL-21 was 16,815.8 Da, suggesting a mature protein beginning at position 30 of the precursor sequence (Gln-Gly-Gln-Asp-Arg-His- vs Gln-Asp-Arg-His-).

5 The present invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

In The Claims

1. An isolated polynucleotide comprising a polynucleotide having the sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11 or 13 or a complementary sequence.
2. An isolated polynucleotide comprising a polynucleotide encoding the amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14 or a complementary sequence.
3. An isolated polypeptide comprising a polypeptide having the sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14.
4. Isolated mature human interleukin-21 (huIL-21) having the amino acid sequence shown in SEQ ID NO: 19.
5. An isolated polynucleotide encoding the mature huIL-21 of claim 4.
6. The isolated polynucleotide of claim 5 having a sequence shown in SEQ ID NO: 18.
7. A vector comprising the isolated polynucleotide of claim 1, 2 or 5.
8. An isolated host cell comprising the vector of claim 7.
9. A process for producing a polypeptide comprising culturing the host cell of claim 8 under conditions sufficient for production of the polypeptide.

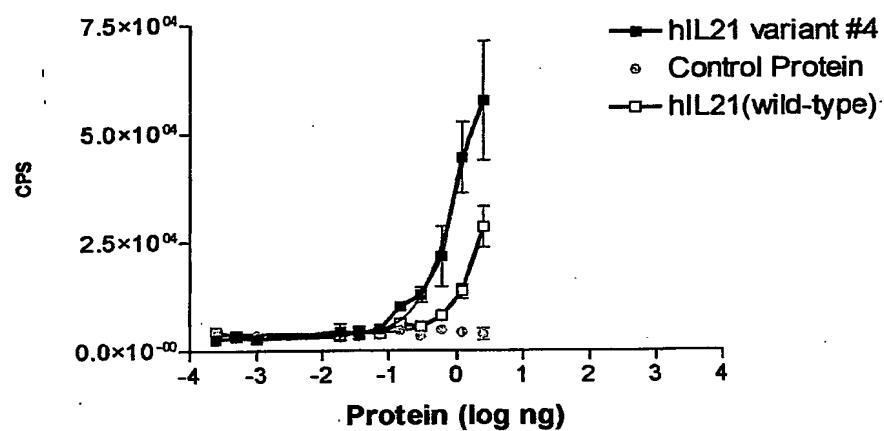


Fig. 1

Native QDRHMIRMRQ LIDIVDQLKN YVNDLVPEFL PAPEDVETNC EWSAFSCFOK AOLKSANTGN

#1	N-----E-----V-----Q-----I-----T-----Q-----
#2	N-----V-----Q-----I-----T-----Q-----
#3	N-----E-----V-----Q-----I-----T-----Q-----
#4	N-----E-----V-----Q-----I-----T-----Q-----
#10A	N-----E-----V-----E-----I-----T-----R-----

Native NERIINVIK KLKRKPSTN AGRROKHRLT CPSCDSYEKK PPKEFLERFK SLLQKMTIHOH LSSRTHGSED S

#1	-----Q-----T-----N-----T-----Y-----Y-----V-----E T
#2	-----Q-----T-----N-----T-----Y-----Y-----V-----E T
#3	-----Q-----T-----N-----T-----Y-----V-----E T
#4	-----Q-----T-----N-----T-----Y-----V-----E T
#10A	-----Q-----T-----N-----T-----Y-----V-----E -

Fig. 2

CEN5029PCT SeqListg 06-04-04.txt
SEQUENCE LISTING

<110> Cunningham, Mark R.; Heavner, George A.; Luo, Jinquan; Song, Xiao-yu;
Centocor, Inc.

<120> Interleukin-21 Analogs

<130> CEN5029 PCT

<150> US 60/427,772

<151> 2003-06-19

<160> 19

<170> PatentIn version 3.2

<210> 1

<211> 393

<212> DNA

<213> Artificial

<220>

<223> Analog #1

<400> 1

aacgatcgcc acatgattag aatgcgttag cttatagatg tgggtatca gctgaaaaat	60
tatgtgaatg acttggtccc tcagtttctg ccagctccag aagatatcga gacaaactgt	120
gagtggtcag ctttacactg tttcagaag gcctaactaa agtcagcaca gacagggaaac	180
aatgaaagga taatccaggt atcaattaaa aagctgaaga ggaaaccacc taccacaat	240
gcagggagaa gaaacaaaca cagactaaca tgcccttcat gtgataccctg tgagaaaaaa	300
ccacccaaag aatacctaga aagataaaaa tcacttctcc aaaagatggt gcatcagcat	360
ctgtcctcca gaacacacgg aagtgaagag acc	393

<210> 2

<211> 131

<212> PRT

<213> Artificial

<220>

<223> Analog #1

<400> 2

Asn Asp Arg His Met Ile Arg Met Arg Glu Leu Ile Asp Val Val Asp			
1	5	10	15

Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Gln Phe Leu Pro Ala		
20	25	30

Pro Glu Asp Ile Glu Thr Asn Cys Glu Trp Ser Ala Phe Thr Cys Phe		
35	40	45

Gln Lys Ala Gln Leu Lys Ser Ala Gln Thr Gly Asn Asn Glu Arg Ile

CEN5029PCT SeqListg 06-04-04.txt
 50 55 60

Ile Gln Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Thr Thr Asn
 65 70 75 80

Ala Gly Arg Arg Asn Lys His Arg Leu Thr Cys Pro Ser Cys Asp Thr
 85 90 95

Tyr Glu Lys Lys Pro Pro Lys Glu Tyr Leu Glu Arg Tyr Lys Ser Leu
 100 105 110

Leu Gln Lys Met Val His Gln His Leu Ser Ser Arg Thr His Gly Ser
 115 120 125

Glu Glu Thr
 130

<210> 3
<211> 393
<212> DNA
<213> Artificial

<220>
<223> Analog #2

<400> 3
aacgatcgcc acatgattag aatgcgtcaa cttatacatg tggttgatca gctgaaaaat 60
tatgtgaatg acttggtccc tcagttctg ccagctccag aagatatcga gacaaactgt 120
gagtggtcag cttttacactg ttttcagaag gcccaactaa agtcagcaca gacaggaaac 180
aatgaaagga taatccaggt atcaattaaa aagctgaaga ggaaaccacc taccacaaat 240
gcagggagaa gaaacaaaaca cagactaaca tgcccttcat gtgataccta tgagaaaaaa 300
ccacccaaag aatacctaga aagatacaaa tcacttctcc aaaagatggt gcatcagcat 360
ctgtccctcca gaacacacgg aagtgaagag acc 393

<210> 4
<211> 131
<212> PRT
<213> Artificial

<220>
<223> Analog #2

<400> 4

Asn Asp Arg His Met Ile Arg Met Arg Gln Leu Ile Asp Val Val Asp
 1 5 10 15

Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Gln Phe Leu Pro Ala
 20 25 30

CEN5029PCT SeqListg 06-04-04.txt

Pro Glu Asp Ile Glu Thr Asn Cys Glu Trp Ser Ala Phe Thr Cys Phe
 35 40 45

Gln Lys Ala Gln Leu Lys Ser Ala Gln Thr Gly Asn Asn Glu Arg Ile
 50 55 60

Ile Gln Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Thr Thr Asn
 65 70 75 80

Ala Gly Arg Arg Asn Lys His Arg Leu Thr Cys Pro Ser Cys Asp Thr
 85 90 95

Tyr Glu Lys Lys Pro Pro Lys Glu Tyr Leu Glu Arg Tyr Lys Ser Leu
 100 105 110

Leu Gln Lys Met Val His Gln His Leu Ser Ser Arg Thr His Gly Ser
 115 120 125

Glu Glu Thr
 130

<210> 5
 <211> 393
 <212> DNA
 <213> Artificial

<220>
 <223> Analog #3

<400> 5
 aacgatcgcc acatgattag aatgcgtgag cttatacatgatg tggttgatca gctgaaaaat 60
 tatgtgaatg acttggtccc tcagttctg ccagctccag aagatatcga gacaaactgt 120
 gagtggtcag cttttacctg ttttcagaag gcccaactaa agtcagcaca gacagggaaac 180 /
 aatgaaaaggta taatccaggt atcaatttaaa aagctgaaga gggaaaccacc taccacaaat 240
 gcagggagaa gaaacaaaca cagactaaca tgcccttcat gtgataccta tgagaaaaaa 300
 ccacccaaag aataacctaga aagattcaaa tcacttctcc aaaagatggt gcatcagcat 360
 ctgtcctcca gaacacacgg aagtgaagag acc 393

<210> 6
 <211> 131
 <212> PRT
 <213> Artificial

<220>
 <223> Analog #3

<400> 6

CEN5029PCT SeqListg 06-04-04.txt

Asn Asp Arg His Met Ile Arg Met Arg Glu Leu Ile Asp Val Val Asp
1 5 10 15

Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Gln Phe Leu Pro Ala
20 25 30

Pro Glu Asp Ile Glu Thr Asn Cys Glu Trp Ser Ala Phe Thr Cys Phe
35 40 45

Gln Lys Ala Gln Leu Lys Ser Ala Gln Thr Gly Asn Asn Glu Arg Ile
50 55 60

Ile Gln Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Thr Thr Asn
65 70 75 80

Ala Gly Arg Arg Asn Lys His Arg Leu Thr Cys Pro Ser Cys Asp Thr
85 90 95

Tyr Glu Lys Lys Pro Pro Lys Glu Tyr Leu Glu Arg Phe Lys Ser Leu
100 105 110

Leu Gln Lys Met Val His Gln His Leu Ser Ser Arg Thr His Gly Ser
115 120 125

Glu Glu Thr
130

<210> 7
<211> 393
<212> DNA
<213> Artificial

<220>
<223> Analog #4

<400> 7
aacgatcgcc acatgattag aatgcgttag cttatagatg tggttgatca gctaaaaat 60
tatgtaatg acttggccc tcagttctg ccagctccag aagatatcga gacaaactgt 120
gagtggtcag ctttacctg tttcagaag gcccaactaa agtcagcaca gacaggaaac 180
aatgaaaagga taatccaggt atcaattaaa aagctgaaga ggaaaccacc taccacaaat 240
gcagggagaa gaaacaaaaca cagactaaca tgcccttcat gtgataccta tgagaaaaaa 300
ccacccaaag aattcctaga aagataaaaa tcacttctcc aaaagatggt gcatcagcat 360
ctgtcctcca gaacacacgg aagtgaagag acc 393

<210> 8
<211> 131

CEN5029PCT SeqListg 06-04-04.txt

<212> PRT
 <213> Artificial

<220>
 <223> Analog #4

<400> 8

Asn Asp Arg His Met Ile Arg Met Arg Glu Leu Ile Asp Val Val Asp
 1 5 10 15

Gln Leu Lys Asn Tyr Ile Asn Asp Leu Val Pro Gln Phe Leu Pro Ala
 20 25 30

Pro Glu Asp Ile Glu Thr Asn Cys Glu Trp Ser Ala Phe Thr Cys Phe
 35 40 45

Gln Lys Ala Gln Leu Lys Ser Ala Gln Thr Gly Asn Asn Glu Arg Ile
 50 55 60

Ile Gln Val Ser Ile Lys Leu Lys Arg Lys Pro Pro Thr Thr Asn
 65 70 75 80

Ala Gly Arg Arg Asn Lys His Arg Leu Thr Cys Pro Ser Cys Asp Thr
 85 90 95

Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Tyr Lys Ser Leu
 100 105 110

Leu Gln Lys Met Val His Gln His Leu Ser Ser Arg Thr His Gly Ser
 115 120 125

Glu Glu Thr
 130

<210> 9
 <211> 393
 <212> DNA
 <213> Artificial

<220>
 <223> Analog #10A

<400> 9		
aacgatcgcc acatgattag aatgcgtgag cttatagatg tggatcgatca gctgaaaaat	60	
tatgtgaatg agttggtccc tgaatttctg ccagctccag aagatatcga gacaaactgt	120	
gagtggtcag cttttacctg ttttcagaag gcccaactac gctcagcaa tacagggaaac	180	
aatgaaagga taatccaggt atcaattaaa aagctgaaga ggaaaccacc taccacaat	240	
gcagggagaa gaaacaaaca cagactaaca tgcccttcat gtgataccta tgagaaaaaa	300	

CEN5029PCT SeqListg 06-04-04.txt

ccacccaaag aattcctaga aagatacaaa tcacttctcc aaaagatggt gcatcagcat	360
ctgtcctcca gaacacacgg aagtgaagag tcc	393

<210> 10
<211> 131
<212> PRT
<213> Artificial

<220>
<223> Analog #10A

<400> 10

Asn Asp Arg His Met Ile Arg Met Arg Glu Leu Ile Asp Val Val Asp		
1 5	10	15

Gln Leu Lys Asn Tyr Val Asn Glu Leu Val Pro Glu Phe Leu Pro Ala		
20	25	30

Pro Glu Asp Ile Glu Thr Asn Cys Glu Trp Ser Ala Phe Thr Cys Phe		
35	40	45

Gln Lys Ala Gln Leu Arg Ser Ala Asn Thr Gly Asn Asn Glu Arg Ile		
50	55	60

Ile Gln Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Thr Thr Asn			
65	70	75	80

Ala Gly Arg Arg Asn Lys His Arg Leu Thr Cys Pro Ser Cys Asp Thr		
85	90	95

Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Tyr Lys Ser Leu		
100	105	110

Leu Gln Lys Met Val His Gln His Leu Ser Ser Arg Thr His Gly Ser		
115	120	125

Glu Glu Ser
130

<210> 11
<211> 399
<212> DNA
<213> Artificial

<220>
<223> Analog #10B

<400> 11 caaggtaacg atcgccacat gattagaatg cgtgagctta tagatgtggc tgatcagctg	60
aaaaattatg tgaatgagtt ggccctgaa ttctgcccag ctccagaaga tatcgagaca	120

CEN5029PCT SeqListg 06-04-04.txt

aactgtgagt ggtcagctt tacctgttt cagaaggccc aactacgctc agcaaataca	180
ggaaacaatg aaaggataat ccaggtatca attaaaaagc tgaagaggaa accacctacc	240
acaaatgcag ggagaagaaa caaacacaga ctaacatgcc cttcatgtga tacctatgag	300
aaaaaaccac ccaaagaatt cctagaaaga tacaaatcac ttctccaaaa gatggtgcat	360
cagcatctgt cctccagaac acacggaagt gaagagtcc	399

<210> 12
<211> 133
<212> PRT
<213> Artificial

<220>
<223> Analog #10B
<400> 12

Gln Gly Asn Asp Arg His Met Ile Arg Met Arg Glu Leu Ile Asp Val
1 5 10 15

Val Asp Gln Leu Lys Asn Tyr Val Asn Glu Leu Val Pro Glu Phe Leu
20 25 30

Pro Ala Pro Glu Asp Ile Glu Thr Asn Cys Glu Trp Ser Ala Phe Thr
35 40 45

Cys Phe Gln Lys Ala Gln Leu Arg Ser Ala Asn Thr Gly Asn Asn Glu
50 55 60

Arg Ile Ile Gln Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Thr
65 70 75 80

Thr Asn Ala Gly Arg Arg Asn Lys His Arg Leu Thr Cys Pro Ser Cys
85 90 95

Asp Thr Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Tyr Lys
100 105 110

Ser Leu Leu Gln Lys Met Val His Gln His Leu Ser Ser Arg Thr His
115 120 125

Gly Ser Glu Glu Ser
130

<210> 13
<211> 399
<212> DNA
<213> Artificial

CEN5029PCT SeqListg 06-04-04.txt

<220>
<223> Analog #4A

<400> 13
caaggtaacg atcgccacat gattagaatg cgtgagctta tagatgtggc tgcgtcgtc 60
aaaaattatg tgaatgactt ggtccctcag tttctgccag ctccagaaga tatcgagaca 120
aactgtgagt ggtcagctt tacctgttt cagaaggccc aactaaagtc agcacagaca 180
gaaaacaatg aaaggataat ccaggtatca attaaaaagc tgaagaggaa accacctacc 240
acaaatgcag ggagaagaaa caaacacaga ctaacatgcc cttcatgtga tacctatgag 300
aaaaaaccac ccaaagaatt cctagaaaga tacaaatcac ttctccaaaa gatggtgcat 360
cagcatctgt cctccagaac acacggaagt gaagagacc 399

<210> 14
<211> 133
<212> PRT
<213> Artificial

<220>
<223> Analog #4A

<400> 14

Gln Gly Asn Asp Arg His Met Ile Arg Met Arg Glu Leu Ile Asp Val
1 5 10 15

Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Gln Phe Leu
20 25 30

Pro Ala Pro Glu Asp Ile Glu Thr Asn Cys Glu Trp Ser Ala Phe Thr
35 40 45

Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Gln Thr Gly Asn Asn Glu
50 55 60

Arg Ile Ile Gln Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Thr
65 70 75 80

Thr Asn Ala Gly Arg Arg Asn Lys His Arg Leu Thr Cys Pro Ser Cys
85 90 95

Asp Thr Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Tyr Lys
100 105 110

Ser Leu Leu Gln Lys Met Val His Gln His Leu Ser Ser Arg Thr His
115 120 125

Gly Ser Glu Glu Thr
130

CEN5029PCT SeqListg 06-04-04.txt

<210> 15
<211> 31
<212> PRT
<213> Homo sapiens

<400> 15

Met Ala Ser Ser Pro Gly Asn Met Glu Arg Ile Val Ile Cys Leu Met
1 5 10 15

Val Ile Phe Leu Gly Thr Leu Val His Lys Ser Ser Ser Gln Gly
20 25 30

<210> 16
<211> 131
<212> PRT
<213> Homo sapiens

<400> 16

Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile Asp Ile Val Asp
1 5 10 15

Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu Phe Leu Pro Ala
20 25 30

Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala Phe Ser Cys Phe
35 40 45

Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn Asn Glu Arg Ile
50 55 60

Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Ser Thr Asn
65 70 75 80

Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro Ser Cys Asp Ser
85 90 95

Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Phe Lys Ser Leu
100 105 110

Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg Thr His Gly Ser
115 120 125

Glu Asp Ser
130

<210> 17
<211> 29
<212> PRT

CEN5029PCT SeqListg 06-04-04.txt

<213> Homo sapiens

<400> 17

Met	Ala	Ser	Ser	Pro	Gly	Asn	Met	Glu	Arg	Ile	Val	Ile	Cys	Leu	Met
1				5				10					15		

Val	Ile	Phe	Leu	Gly	Thr	Leu	Val	His	Lys	Ser	Ser	Ser
					20			25				

<210> 18

<211> 399

<212> DNA

<213> Homo sapiens

<400> 18

caaggtcaag	atcgccacat	gattagaatg	cgtcaactta	tagatattgt	tgatcagctg	60
aaaaattatg	tgaatgactt	ggtcgcctgaa	tttctgccag	ctccagaaga	tgttagagaca	120
aactgtgagt	ggtcagctt	ttcctgtttt	cagaaggccc	aactaaagtc	agcaaataca	180
ggaaaacaatg	aaaggataat	caatgttatca	attaaaaagc	tgaagaggaa	accacccccc	240
acaaaatgcag	ggagaagaca	gaaacacaga	ctaacatgcc	cttcatgtga	ttcttatgag	300
aaaaaaaccac	ccaaagaatt	cctagaaaga	ttcaaattcac	ttctccaaaa	gatgattcat	360
cagcatctgt	cctctagaac	acacggaagt	gaagattcc			399

<210> 19

<211> 133

<212> PRT

<213> Homo sapiens

<400> 19

Gln	Gly	Gln	Asp	Arg	His	Met	Ile	Arg	Met	Arg	Gln	Leu	Ile	Asp	Ile
1				5				10				15			

Val	Asp	Gln	Leu	Lys	Asn	Tyr	Val	Asn	Asp	Leu	Val	Pro	Glu	Phe	Leu
					20			25			30				

Pro	Ala	Pro	Glu	Asp	Val	Glu	Thr	Asn	Cys	Glu	Trp	Ser	Ala	Phe	Ser
					35		40			45					

Cys	Phe	Gln	Lys	Ala	Gln	Leu	Lys	Ser	Ala	Asn	Thr	Gly	Asn	Asn	Glu
					50		55			60					

Arg	Ile	Ile	Asn	Val	Ser	Ile	Lys	Leu	Lys	Arg	Lys	Pro	Pro	Ser
					65		70		75	80				

Thr	Asn	Ala	Gly	Arg	Arg	Gln	Lys	His	Arg	Leu	Thr	Cys	Pro	Ser	Cys
						85		90			95				

CEN5029PCT SeqListg 06-04-04.txt

Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Phe Lys
100 105 110

Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg Thr His
115 120 125

Gly Ser Glu Asp Ser
130